AUTOMATED SOYBEAN CYST NEMATODE EXTRACTION UNIT

BY

ALLANTÉ V. WHITMORE

THESIS

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Master's Committee:

Associate Professor Kaustubh Bhalerao, Chair
Associate Professor Kris Lambert
Associate Professor Tony Grift

ABSTRACT

The soybean cyst nematode (SCN) is a plant parasitic roundworm of soybeans responsible for an estimated $0.7-1.2 billion reduction in annual yield loss. In order to manage yield loss due to SCN, soybean producers employ a nematode management plan which utilize extensive soil sampling to assess SCN population density. Even though the nematode management practices exist, they require greater spatial and temporal resolution in SCN field counts. Currently, SCN extraction from soil samples is time consuming and highly laborious. This thesis produced a design and prototype of an automated extraction unit in order to simplify the soybean cyst and egg extraction process. The proposed automated extraction process will increase the recovery rate while consolidating nematode extraction steps eliminating much of the manual labor in the process. In order to improve extraction efficiency, the design maximized automation use, compacted unit size, used inexpensive materials, and offered the option to scale the unit. Prototyping established the technical feasibility of the unit. The automated unit will provide a more efficient SCN extraction method for monitoring the SCN population.

To Mr. Herbert Whitmore, my grandfather.

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Chapter 1

BACKGROUND

Soybean cyst nematodes (SCN) or *Heterodera glycines* is believed to be first reported on American soil in 1954 in North Carolina (Gange, 2005). The parasite was introduced when soil was imported from Japan in the late 1800s to obtain nitrogen-fixing bacteria (Davis, 2005). These pests have quickly spread and can be found on most soybean producing areas in the Midwest. SCN is the leading soybean pathogen, responsible for an estimated $0.7-1.2 billion reduction in annual loss yield due to the feeding habits of nematodes on soybean plant roots (Davis, 2005).

There are multiple stages of the SCN life cycle: the egg, juvenile stages 1, 2, 3, 4 (J1-J4) and the adult male and female (see Figure 1.1). SCN typically completes its life cycle in three to four weeks. Eggs develop into J1 inside the eggs, then hatch as J2 which are motile and capable of infecting the soybean root to feed and grow. As the SCN feed, they molt three times increasing in size after each molt. As a female SCN matures her body swells, rupturing the root. Mature male nematodes grow and leave the root to find females to mate. Female nematodes remain attached to the root and continue to feed and produce eggs inside and outside of her body, producing approximately 200-600 eggs. The female eventually dies resulting in a brown lemon shaped cyst holding the unhatched eggs (Davis, 2005). The cysts can be seen on roots with the naked eye.

The SCN damage roots, reduce water uptake and interfere with nodulation by nitrogen fixing bacteria, thus reducing yield (Bissonnette, 2012; Morrison, 2014). Common symptoms caused by SCN infestation include stunted growth and yellowing leaves, which is easily mistaken for nutritional stress. The entire life cycle takes place underground, thus in order to determine SCN infection levels soil must be collected from infected sites. The soil samples are processed to extract and

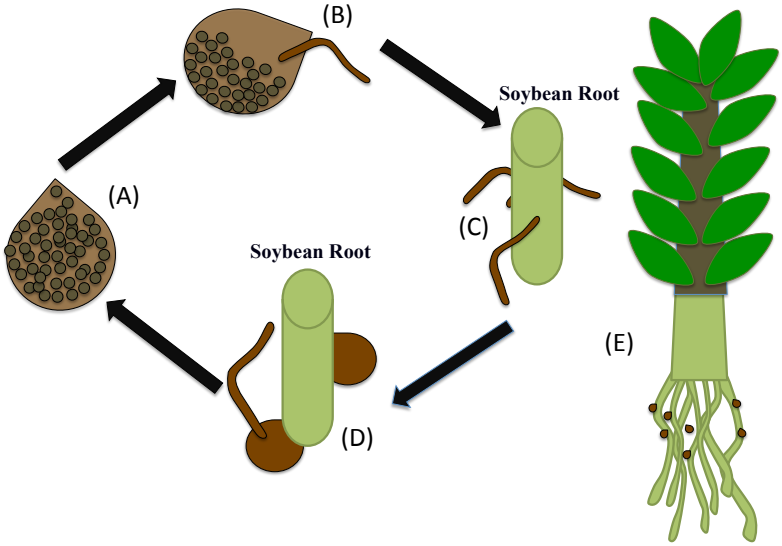

Figure 1.1: This figure illustrates the SCN life cycle. (A) shows the eggs in J1, unhatched juvenile stage. (B) shows J2, the hatched juvenile stage. (C) J2 nematodes are motile and reach the soybean roots for feeding. The nematode molt three more times then reach adulthood. (D) The male nematode then mates with the female, therefore fertilizing the eggs. (E) SCN is found solely on the root of the soybean plant.

count the number of eggs. The SCN egg counts are used to make decision about nematode management practices including crop rotation, nematicides, or the use of SCN resistant soybean varieties.

1.1 SCN Control

Once SCN is introduced into a field, it is currently impossible to eliminate, but yield loss can be reduced the implementation of SCN control strategies. The common approaches to controlling SCN are (1) rotating the field with non-host crops, (2) rotating to SCN resistant soybean varieties, (3) applying nematicides or (4) implementing integrated pest management methods. Non-host crop rotation is currently the most effective approach to decrease SCN population in the northern region of America (AGVISE, 2005). Non-host crops typically planted include small grains, corn, flax, sugar beet, potato, and sunflower (AGVISE, 2005; Neher, 2001). Some poor host crops like alfalfa, red clover and peas (AGVISE, 2005) can also be planted.

Although crop rotation is an effective method to control SCN, many factors must be considered. According to a study done at the University of Minnesota, crop rotation depends upon geographic location, crop species grown, and the numbers of years crop rotation have been in place (Warnke *et al.* , 2006;). Also, SCN resistant soybean cultivars must be rotated to avoid SCN adaptation (Niblack, 2005). It can take over five years for SCN populations to decrease in northern soybean growing areas due to the overwintering ability of the SCN cyst (Morrison, 2014; Bissonnette, 2012; AGVISE, 2005). Crop rotation is a satisfactory management approach, but the variability in results indicate room for improvement.

Nematicides are chemical pesticides that kill SCN in the active J2 phase in soil when searching for roots of soybeans (Gowen, 2008; Trevathan & Robbins, 1995). The heterogenous nature of SCN and high toxicity of nematicides that have caused groundwater contamination in the past (Gowen, 2008), make application economically and environmentally unjustifiable (University of Minnesota Extension, 2014). Integrated pest management (IPM) is an approach that has gained some traction in the past few years. It involves exploiting natural antagonists of SCN in the soil

ecosystem (University of Minnesota Extension, 2014). As an example, according to Chen et. al, some soybean fields in Minnesota experience more than 60% of second stage juveniles being parasitized by fungi present in the soil. IPMs aim to suppress SCN populations, resulting in decreased need for crop rotation planting. Since SCN cannot be eliminated, only controlled, literature stresses that soybean producers monitor the SCN population density via soil testing. (AGVISE, 2005; Niblack, 2005; Morrison, 2014; Gowen, 2008; Bissonnette, 2012). Soil testing provides the magnitude of the SCN burden which then informs the appropriate SCN management option.

1.2 The Economics of Soil Testing

Soil testing for SCN is available as an option to farmers from soil testing companies; soil analysis services typically offered include pH, organic matter, nutrients, nitrates, metals and SCN assays. As noted in Section 1.1, soil testing to determine SCN population is the only way to diagnose and monitor SCN population in a field (Barker & Campbell, 1981; Bissonnette, 2012; Davis, 2005; Ingham, 1994; McSorley, 1987; Perry, 1951). There is a small group of national soil testing companies, in addition to university extension offices that provide soil analysis services. In order to achieve an accurate estimate of SCN population in a field, one sample, consisting of 20-30 subsamples or cores, should represent no more than 10 acres (Morrison, 2014), or 2-3 subsamples per acre. In areas with visible damage, which corresponds to greater than 30% yield loss due to SCN, one composite sample should be taken for the problem area.

SCN population is distributed heterogeneously; if two soybean plants are neighboring, one may be infested with SCN and the other might not. Therefore it is important to have adequate spatial resolution in sampling so proper management of SCN can occur. Table 1.1 provides the current cost to test soil for SCN population density. The high cost of SCN population assessment is a disincentive for soybean producers. As a result, producers typically submit one composite of the entire soybean producing area. Composite soil sampling, while cost effective, does not provide the required spatial or temporal resolution to make appropriate

US average price per acre for maximum soybean yield (USDA, 2013)	$128/acre
15% yield loss due to SCN	$19.20/acre
20% yield loss due to SCN	$25.60/acre
Cost of SCN soil testing	$20-22/sample
Suggested sampling size	20-30 subsamples per 10 acres
Cost of SCN testing per sample	$0.73/sample

Table 1.1: Comparative look at SCN testing and soybean production profit. Prices on soybean production are from the USDA National Agricultural Statistics Service.

SCN management decisions. Soil testing for SCN population density can improve with more samples comprised of smaller, more concentrated sampling areas. If a producer can obtain more than one sample per acre to be tested, nematode management practices can be applied more accurately. To illustrate the incentives provided when sampling area is reduced, consider a field that has reached high-level SCN infection (over 16,000 eggs per 100cc of soil) in a specific 4-acre section. If the soybean producer collected multiple unblended soil samples, the likeliness of collecting SCN from the soil will increase. Once the samples are processed at a soil testing lab, the location of SCN infection would be revealed. Additionally, this would allow the producer to apply SCN management efforts more accurately. Conversely, soil testing a composite sample could reveal a medium or low level as the SCN population density. This is because the highly infected location is 8-12 subsamples of 20-30 samples. Accordingly, the SCN management plan would provide a sweeping control method that may not be aggressive enough to suppress the SCN present in the concentrated location. Overall, soybean producers can benefit from more samples comprised of smaller, more concentrated sampling areas. However, SCN extraction requires extensive time, labor, or capital.

Additionally, soil is not allowed to be transported across state lines without permits (Federal Regulations, 2014), which are granted after passing a lab inspection by a USDA inspector. This process can take up to 90 days to receive the permits that expire after three years (Federal Regulations, 2014). This obstacle

has led many national soil testing labs to either downsize to serve one state, or expand and open soil testing sites in each state. The USDA regulations and the inability of the SCN testing process to scale effectively contribute to the high cost of SCN testing.

1.3 Objective

The objective of this project is to design and construct a prototype for an automated SCN extraction unit to improve the assessment of SCN population. Current nematode extraction processes are completed in six steps: sample collection, soil preparation, cyst extraction, clean up, egg extraction, and egg counting. Sample collection obviously takes place in the field, therefore university extension offices and soil testing companies have provide instructions on how to collect samples. The instructions are used to ensure the producers gather an adequate sample and to reduce soil variability. Unfortunately, the samples sent to testing sites vary in adherence to the instruction or soil types are so dense that soil sample preparation is critical for proper diagnosis. Cyst extraction has been executed in many different ways but each have optimal conditions that result in a desirable recovery rate. Egg extraction is easy to achieve, but current techniques reveal time inefficiency and additional manual labor. Egg counting is the most time consuming step. Testing sites are responsible for completing the SCN extraction in order to physically count the eggs for diagnosis. The proposed automated extraction process will increase SCN extraction uniformity while consolidating nematode extraction steps without virtually any manual labor.

1.3.1 Design Specifications

Traditional extraction methods are completed in six steps soil field collection, soil preparation, cyst extraction, cleaning, egg extraction, and egg counting. The proposed design will introduce a new SCN extraction process and intends to improve extraction efficiency and uniformity. In order to achieve this goal, these design objectives have been developed:

- A new SCN extraction process that can consolidates extraction steps therefore reducing time
- Reduction in manual labor associated with extraction by automating the entire process
- Scalability in design of the extraction unit, meaning that increasing the sample processing throughout comes at a smaller marginal cost in equipment and labor
- Equipment and process designed to have the footprint of a typical lab bench in order to save space
- Low cost design to allow soil testing companies to acquire multiple units for operations in different states and so remove the need to transport soil across state lines
- Compatibility in design so that the SCN extraction unit can be seamlessly integrated into the soil testing labs' existing setup and workflow

Chapter 2

SOYBEAN CYST EXTRACTION

The SCN characterization needs for a soybean producer are different from the nematode researcher. The soybean producer requires constant monitoring and quantification of SCN numbers, while the objectives of nematode research are broadly divided into exploratory and ecological surveys. Exploratory surveys look at the population density of a particular nematode and do not require a high level of accuracy. Ecological surveys characterize the different taxa of nematodes, thus need a high degree of accuracy which requires a technique that produces a higher cyst recovery (Fields *et al.* , 1955). Soil testing sites focus on SCN infestation as its related to population density, so this review will primarily focus on exploratory survey extraction methods relevant to the design and construction of my SCN extractor prototype.

2.1 Baermann Funnel

The Baermann funnel technique is the most commonly used method for nematode extraction. Figure 2.1 illustrates how soil samples are placed on 2-ply tissue onto a wire mesh inside of a plastic funnel with water. After 24 to 96 hours (McSorley & Walter, 1991) the nematodes can be found in the water as they have traveled through the soil. This technique is common because it is inexpensive and highly efficient for vermiform nematode collection and producing a clean nematode sample. Despite these advantages, the soil type poses some limitations. The Baermann technique is effective to extract nematodes from sandy and loam soil, but recovery decreases when nematodes are extracted from peat soil (Harrison, 1976). The Baermann Funnel technique is not ideal for SCN because cysts are

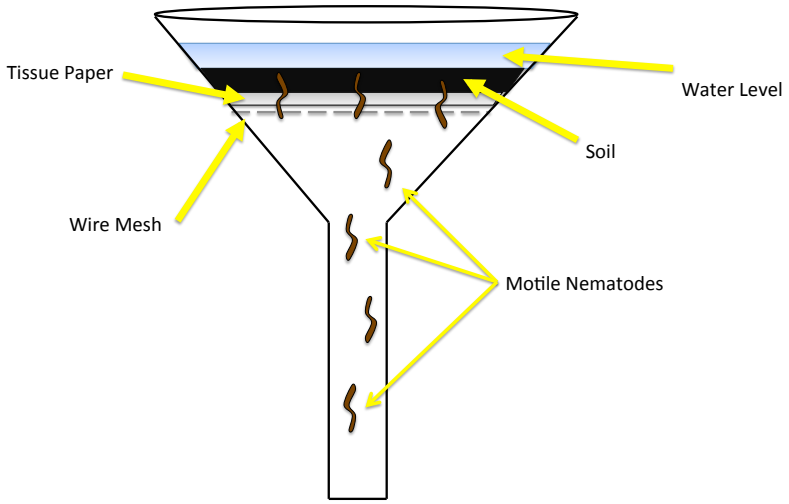

Figure 2.1: An illustration of the Baermann funnel technique. Motile nematodes collect at the bottom of the funnel. This method is not effective due to the non-motile nature of SCN.

non-motile Fields *et al.*, 1955). Additionally, soybean producers send soil samples to testing labs so soil handling is variable and there is no guarantee all nematodes are alive when they are sent. Furthermore, soil samples are typically processed within a week which can also compromise nematode vitality. Although the Baermann funnel technique is popular in nematology, it is not sufficient for the project objectives.

2.2 Cobb Sieving and Decanting technique

Sieving and decanting, shown in Figure 2.2 is a very common extraction method for cyst nematodes that exploits the shape and size of nematodes for extraction (Harrison, 1976). A soil sample, approximately 500 grams or less, is placed in a bucket then filled up three-fourths full with water. Soil clumps are homogenized by hand, then the solution is vigorously stirred for 30-45 seconds. The sample is then decanted over a 10 mesh sieve and rinsed off into a second bucket.

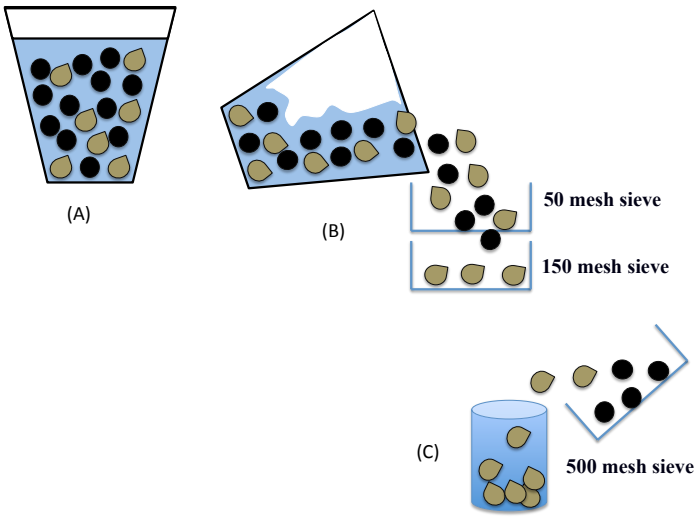

Figure 2.2: The Cobb decanting and sieving method is very simplistic but yields a tolerable (50%) egg recovery. (A) The soil sample is mixed to release cysts to float to the top of the bucket. (B) The sample is decanted over a stack of sieves, with cysts landing in the bottom sieve. (C) The final sample, containing only cysts and fine soil particles are transported to extract the eggs.

The second bucket is mixed vigorously and the sample is decanted over a stack of sieves and the contents of the sieves are washed into beakers. The beaker contents are then poured into counting dishes. Without sufficient soil homogenization prior to extraction, sieves are often plugged with soil. The cyst recovery rate has been found to improve by pouring the same water through each screen multiple times with the screen held at a 45° angle. This extraction method is advantageous because nematodes can be observed in a few minutes and most species of nematode are recovered in good condition (Frederick & McSorley, 1991; Ingham, 1994; McSorley & Frederick, 2004). Many laboratories use a modified version of this procedure (Gange, 2005) but it is too time consuming to process large volumes of samples. Multiple sources (Ferris, 1987; Nagy, 1996; Gange, 2005) find that the cysts are difficult to detect due to remaining organic debris.

2.3 Centrifugation method

Centrifugation is a very common extraction method that was created by Caveness and Jensen in 1955. This technique accomplishes (1) the isolation of nematode eggs, (2) recovery of a larger portion of nematodes, and (3)removes extraneous matter from the nematode sample(Fields *et al.* , 1955). Samples are sifted through a 20-mesh sieve into a bucket with water to eliminate large debris. The sample is then vigorously stirred and allowed to settle for one minute. The supernatant, which holds the nematodes, is poured over a 400 mesh sieve held at 45° angle. The residue left on the 400 mesh sieve are backwashed through a glass funnel into a tube and centrifuged. The supernatant is decanted and replaced with a 2.5 molar sucrose solution, mixed, and centrifuged for one minute. This results in soil compacted at the bottom of the centrifugation tube and the remaining nematodes and cysts are suspended in the sucrose solution. The nematodes are then transferred into a 500 mesh sieve submerged in water to rinse the nematodes off, and then placed into a counting dish or vial (see Figure 2.3). The method takes approximately twelve minutes to complete and recovers both live and dead nematodes. Centrifugation is also advantageous because the recovery rate for nematode extraction is constant with any soil type (Ingham, 1994). Even though

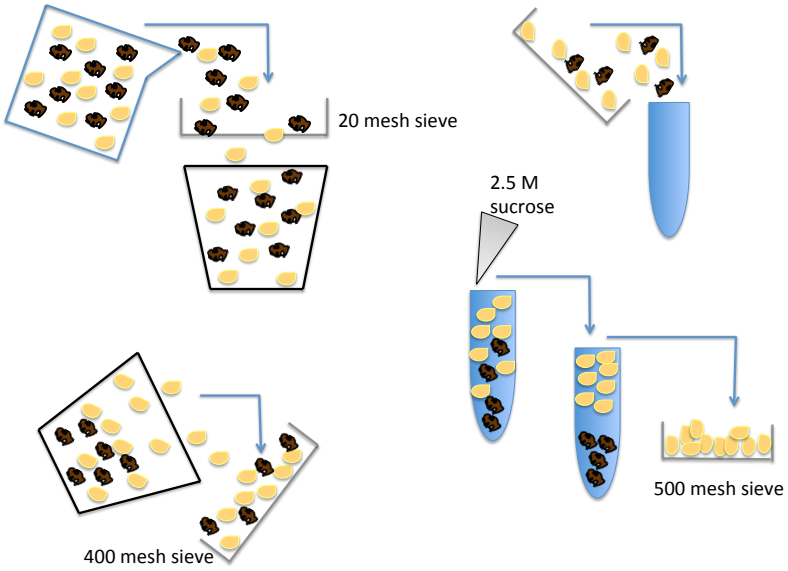

Figure 2.3: Diagram of the sucrose centrifugation nematode extraction technique. Sucrose centrifugation allows for the extraction of nematodes in every stage of the life cycle.

there are advantages, this technique is very laborious since all of the steps are completed manually beside centrifugation. Also, centrifuges can cost upwards of $10,000, thus this technique is not suitable for all soil testing labs especially those who process large volumes of samples.

2.4 Fenwick can method

The Fenwick can (see Figure 2.4) was originally introduced in 1946 as semiautomatic nematode extraction unit. A 20 cm diameter funnel is placed on top of the Fenwick can with an outlet that overlaps a stack of wet mesh sieves (Ingham, 1994; Riggs *et al.* , 1997). The soil sample is placed on a 20-mesh sieve and washed through the funnel and into the can under a strong jet of water. Cysts and organic materials rapidly overflow into the collar and pass down into the collecting sieves (Ingham, 1994; Riggs *et al.* , 1997). The debris is washed off to recover the cysts, averaging an approximate 70% of cysts in the soil sample (Riggs *et al.*

, 1997). The Fenwick can is still in use, because it a method for extraction, but not used as commonly as Cobb decanting sieving or the Baermann method. By introducing the semi-automatic feature cyst recovery increased, but the Fenwick can agitation inlet often plugs with soil.

2.5 Elutriation method

Commonly found in large nematology labs, the elutriator is a semiautomatic nematode extraction machine. The soil sample (250-500g) is poured into one of several steel funnels where the sample is agitated with water and air, which cause an overflow of particulate materials containing cysts over a coarse 40 mesh sieve to catch large organic matter. The remaining filtrate is poured into a sample splitter. The subsamples are directed onto a 325 or 340 mesh sieve stacked atop a 400 mesh sieve that holds the cysts and fine soil particles. A shaker then agitates the 400 mesh screen to release soil plugs. Lastly, the subsamples are processed by sucrose centrifugation or Baermann funnel techniques to extract cysts. The elutriation method takes approximately four minutes and the equipment can easily be cleaned (Ingham, 1994; Ferris, 1987; Frederick McSorley, 1991) automatically. It is also noted for relatively consistent efficiency with reduced variability between operators (Riedel Thistlethwayte, 1969). Elutriators are custom built therefore range between $10,000-$20,000, in addition to the relatively large amount of space needed to house the machine (Ingham, 1994). The elutriator must also be closely monitored to ensure that soil samples with higher clay or silt are well mixed and do not remain at the bottom of the funnel causing soil plugging.

2.6 Contemporary extraction

Smiley (2012)presented a fluidizing column as a low cost alternative to extract SCN. The column was designed for ecological assays. This fluidizing column was intentionally designed as a modified Fenwick can and cost $253 to construct two columns. It was found to extract more efficiently than a Fenwick can with an

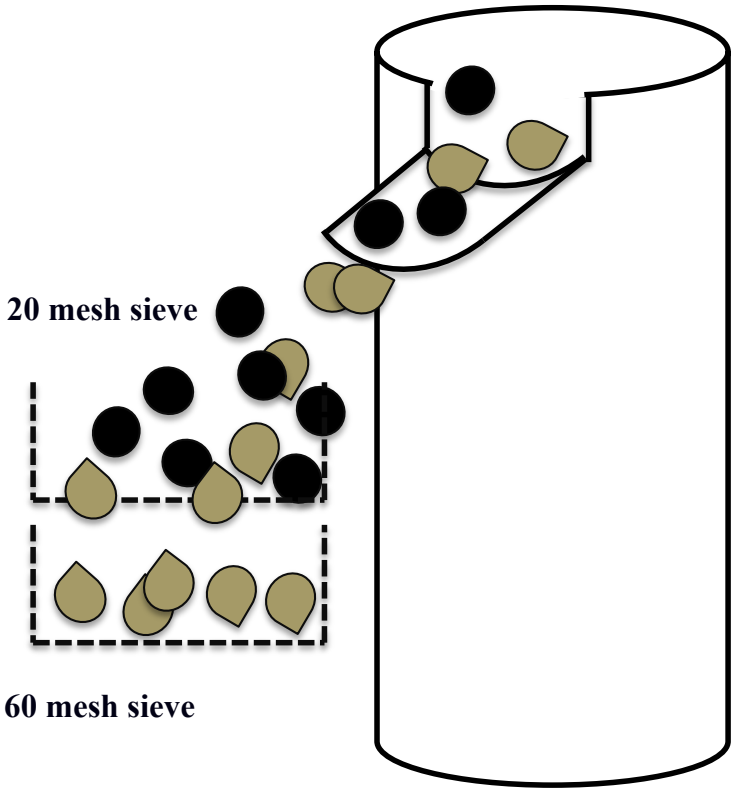

Figure 2.4: The Fenwick can is a simple, semiautomatic nematode extraction unit. The water and air inlet agitates a soil sample to suspend cysts and fine soil in water that overflows over a stack of sieves.

elutriation step. The primary drawback is the longer counting time intervals due to smaller sample capacity. An emerging method (Goto *et al.* , 2011) uses real time polymerase chain reaction primers that are designed for quantitative purpose specific to different plant parasite nematodes. This innovative technique is useful for ecological assays, appropriately using the Baermann funnel technique, which is not suitable for SCN, as noted before.

2.7 Egg Extraction

SCN extraction techniques discussed above provide the sample that contain cysts which hold the eggs. However, the cysts only poorly predict infestation levels. To accurately quantify the SCN burden, the number of SCN eggs within the cysts must be counted. Cysts can easily be crushed mechanically with very little force. Faghihi and Ferris (2000) emphasized a reliable and quick process of grinding cysts against a 60-mesh sieve to release eggs from cysts. A cyst crushing chamber is built with two PVC pipes holding a 60 mesh sieve between them. Then a plunger is constructed with a rubber stopper attached to a drill press. This method is commonly found in laboratories that process large volumes of samples but are typically built in the lab. The chamber is simple to build, but unable to last more than a month under constant operation (Colgrove, 2014). Research labs that process smaller volumes of soil use a rubber roller against a mesh sieve to crush cysts. This is equally effective as the method discussed by Faghihi.

2.8 Summary of drawbacks with current extraction techniques

Soil type, manual labor, time, and cost are factors for consideration when choosing a technique to achieve optimal extraction efficiency. Present techniques each achieve the objective but posses a unique set of trade-offs. Soil restrictions are especially challenging to overcome due to the small size of SCN cysts. Soil homogenization can reduce, but will not eliminate soil clogging from occurring. Soil

type heavily impacts extraction efficiency, so there is a need for a process that will be effective over a wide range of soil types without increasing sample processing time. Additionally, current processes present sample limitations (i.e., only four samples are processed at a time) that also reduce extraction efficiency. Being able to process multiples samples at one time would greatly improve productivity. Manual labor has been reduced by the creation of the elutriator, but the initial costs can be a deterrent especially when soil testing labs would need an elutriator for each testing location. On the other hand, if soil testing labs continue to extract SCN manually, they will need to hire more operators to continue extracting SCN in a timely manner. There is a lack of research regarding extraction techniques and Riggs *et al.* (1997) concluded their article urging efforts be made to improve extraction methods. This presents an opportunity for the development of a new process that can achieve a SCN cyst and egg extraction that increases recovery rate and is time, labor and cost efficient, but has consistent recovery rate.

Chapter 3

EXTRACTION PROCESS AND UNIT DESIGN

High SCN extraction efficiency for the purpose of this paper is determined by the following factors: low sampling time, low cost, low labor intensity, high robustness to soil, and high cyst recovery rate. None of the current extraction methods satisfy all these factors, thus the proposed SCN extraction process and design. In order to improve extraction efficiency the design maximized automation use, compacted unit size, used inexpensive materials, and offered the option to scale the unit. When designing the new extraction unit design both the process and design adapted to accomplish the key objectives of the project. The proposed process, shown in Table 3.1, aimed to consolidate soil homogenization, cyst and egg extraction, and cleaning through design. The design of the extraction unit was sketched in AutoCad Inventor 2012.

3.1 Elutriator

Cyst extraction recovery rate varies by extraction technique. The approach used to develop the cyst extraction component was to utilize the extraction technique that will improve SCN cyst recovery rate, namely elutriation and decanting and sieving. The elutriator was chosen to be added to the extraction process because of the 70-75% cyst recovery rate and four minute cyst extraction time. Although elutriators are advantageous to cyst extraction existing elutriator design occupy too much space. The proposed elutriator dimensions were chosen to be 200 mm in height, upper base diameter of 170 mm, and lower base diameter of 70 mm. The elutriator will still maintain its shape of a frustum of a right circular cone but the diameters have increased in order to accommodate the decreased height. The

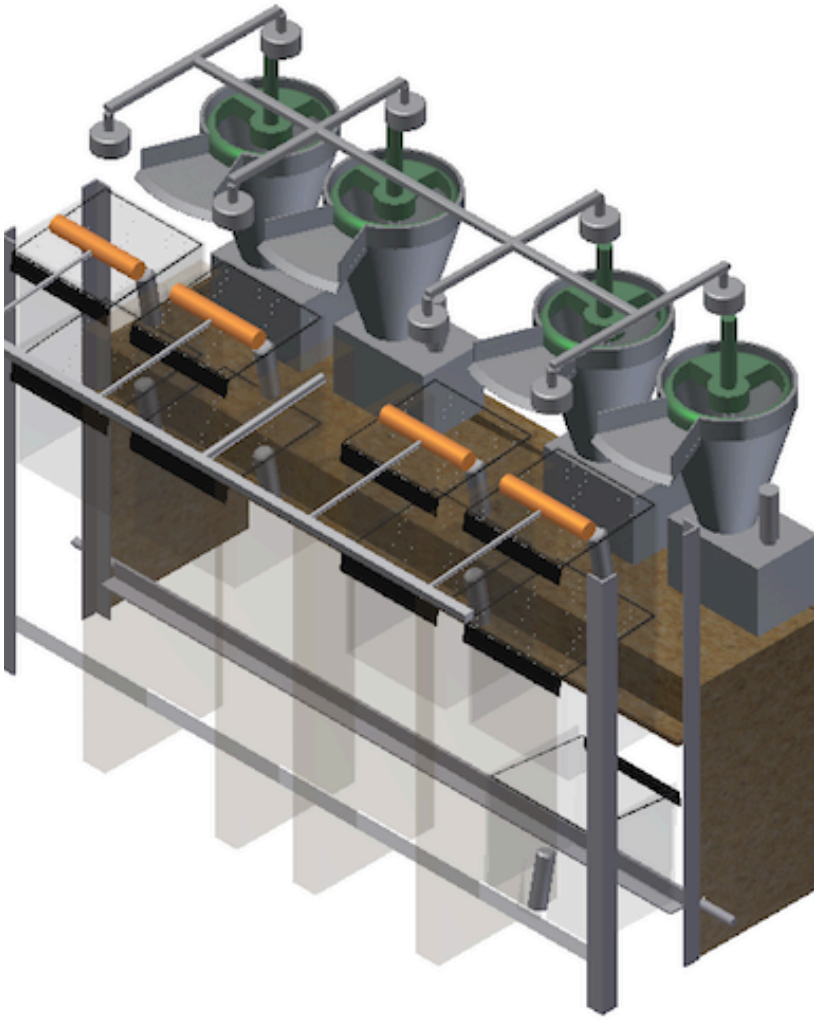

Figure 3.1: The proposed soybean cyst nematode extraction unit.

Table 3.1: Proposed SCN Extraction Process

Elutriation
Elutriator seal is closed Soil sample is deposited in to elutriator. Overhead water inlet inputs 1-2 L of water into elutriator. Soil and water overflow into the extraction channel. Mixing bar and inlet commence to process sample releasing cyst from soil. Elutriator seal is opened disposing soil sample.
Egg extraction
Cysts collect on the surface of the top extraction box. Roller crushes the cysts releasing eggs Overhead water inlet turned on to transport eggs and debris the the second extraction box. Second sieve collects any remaining organic matter from eggs. Third sieve collects eggs and transports them to the collection chamber.
Cleaning
Overhead water inlet and elutriator inlet clean the elutriator. Conical jet sprays clean extraction boxes.

proposed elutriator can still accept a 100 cc sample of soil which is the amount of sample volume commonly processed for cyst extraction. A shower head is placed above the elutriator to provide 2-3 L of water needed to sufficiently release cysts from soil.

Currently, most labs homogenize soil because field samples condition vary when they reach a soil testing site. Soil homogenization is important because it reduces the recovery rate variability due to soil type. Incorporating a mixing bar to elutriation would help to address the soil variability. The mixing bar is housed within the rubber cover used to contain spills during elutriation and will homogenize the soil via an axial flow impeller. Axial flow impellers are very useful in mixing solid-liquid suspensions such as the water and soil, because they will prevent solid particles from settling at the bottom of the elutriator. An inlet is positioned below the mixing bar to assist in floating cysts to the outlet of the elutriator. Inside of the elutriator there is a 10 mesh to prevent large soil clumps from reaching the extraction boxes.

Traditional elutriators are built to accept, process, and dispose of soil samples.

Accordingly, a lever mechanism was designed to automatically open and close a seal at the base of the elutriator. The mechanism is closed during elutriation and opened to transport the soil sample into a disposal system. The mechanism removes the bulk of the sample waste but additional cleaning is necessary to avoid accumulation. The overhead water inlet will rinse the elutriator, removing the remaining soil from inside the elutriator.

3.2 Extraction Box

Cobb sieving and decanting is present in most of the techniques used to extracts cyst with the purpose of improving cyst recovery rate and sample cleanliness. Meshes physically collects the cysts and eggs on its surface. Meshes can be framed multiple ways and maintain functionality. Typical sieves are round in shape but do not properly serve the objectives of this proposed unit design. An extraction box frame the meshes to extract cysts and egg efficiently. The mounted meshes are positioned on an angle because literature suggests that holding sieves on an angle reduce soil plugging and improve cyst recovery (Perry, 1951). The 15° slope was chosen to facilitate debris traveling to the disposal system and hold the cysts and eggs exclusively.

The extraction boxes are set up to resemble sieve stacks. The design of the extraction channel was critical to ensure the eggs and cysts were captured on the meshes. Once elutriation is complete the overflow from the elutriator carries cysts onto the first box, a 100-mesh screen, where the cysts are crushed to release the eggs. The eggs, soil particles, and water fall through the first box where the second box removes more soil particles or any extraneous matter. The third box is mounted to the unit on a pivot to allow for motion throughout the proposed extraction process. During elutriation the box is tipped forward to direct debris and water into the unit disposal system. Once elutriation is complete and egg extraction commences the box is automatically aligned with the other extraction boxes. The eggs, once released, fall through the top two extraction box meshes to the final extraction box framing 400 mesh to collect the eggs. Once egg sample collection is completed, the extraction box is tipped backward to transfer the egg

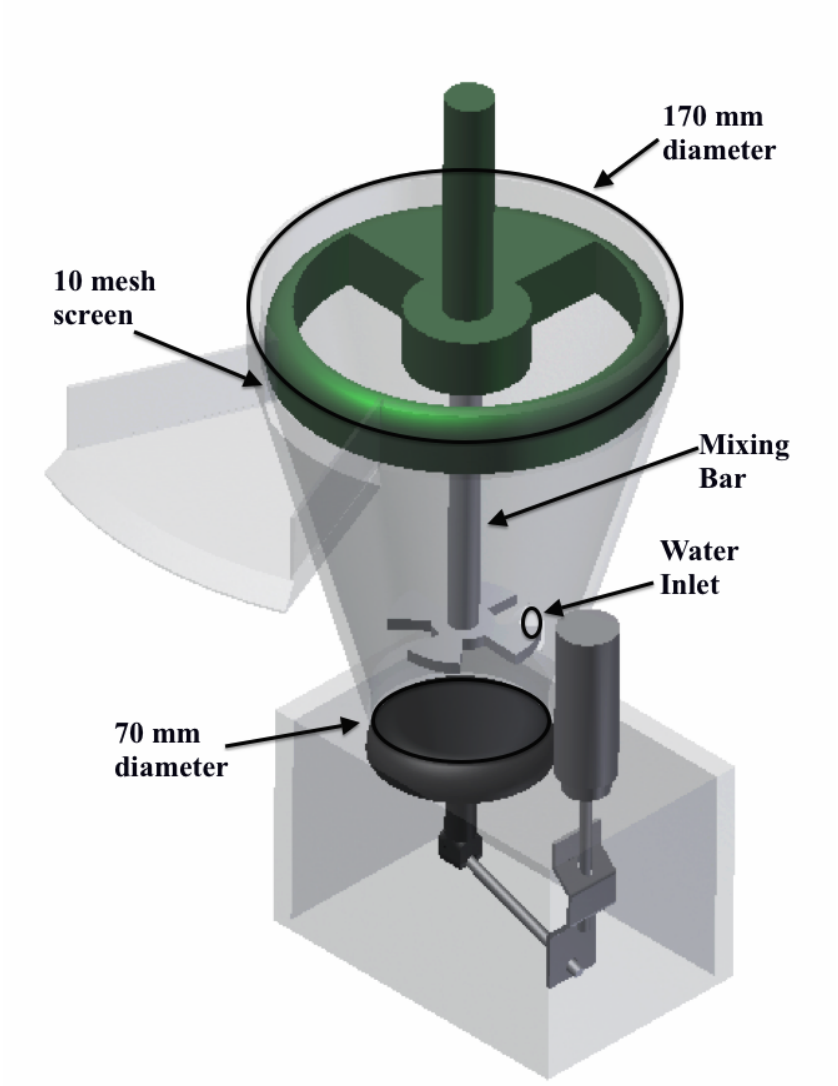

Figure 3.2: The proposed elutriator.

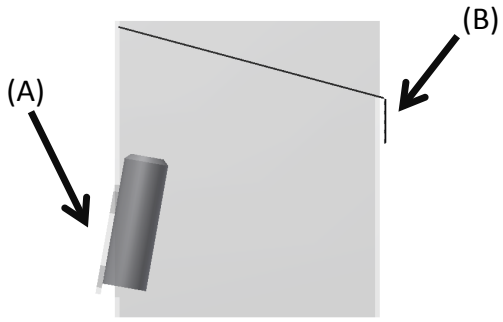

Figure 3.3: Side view of the proposed extraction box. (A) The dark gray cylinder on left side of the box denote the conical jet spray that cleans the extraction box after eggs have been extracted. (B) The overhanging lip facilitates the flow of soil to the disposal system.

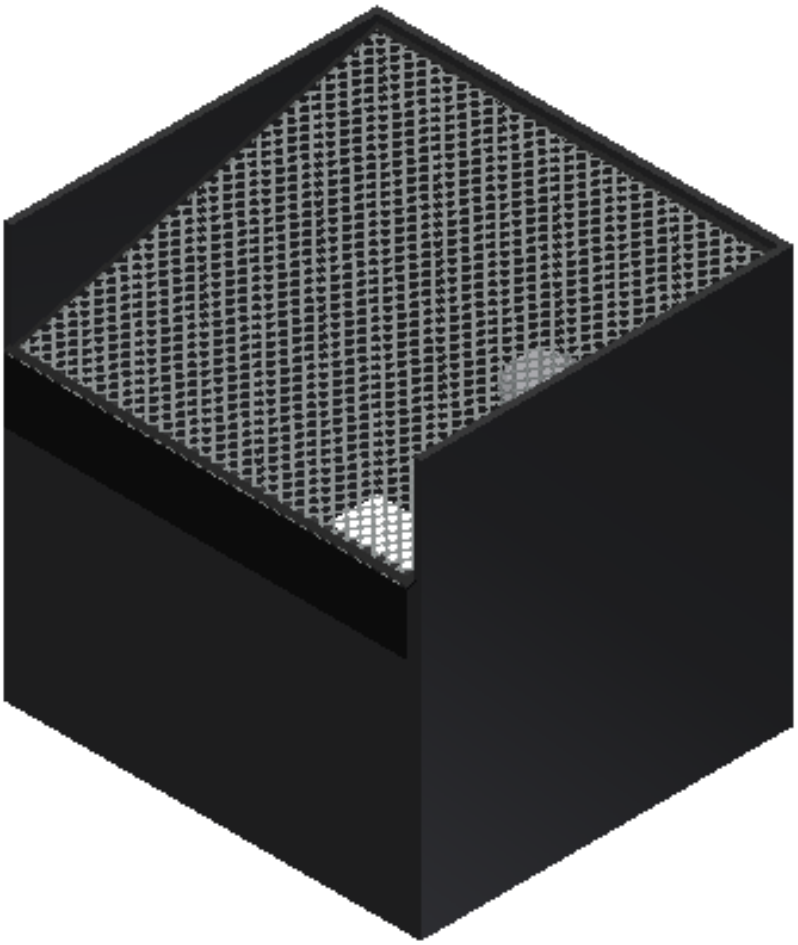

Figure 3.4: Angle view of the proposed extraction box.

sample into a sample collection chamber.

Lastly, all extraction methods require some level of cleaning,which is completed manually. The cyst extractor cleaning step unnecessarily diverts time from processing more samples, impacting productivity. The proposed design introduced self-cleaning into the unit design to decrease time and curb troubleshooting that may arise from soil plugging. Conical jet sprayers are inside every extraction box, rinsing box the box and the mesh, therefore moving debris to the disposal system to reduce soil plugging.

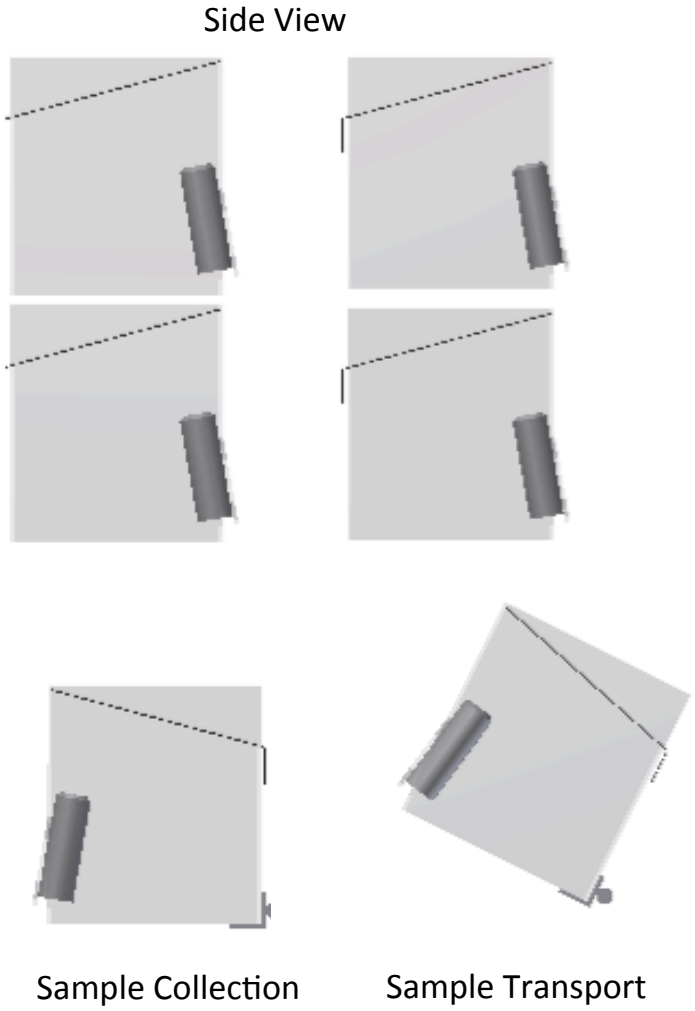

Figure 3.5: Side view of the extraction channel in the position to collect the egg sample, then transport the sample to a collection chamber.

Chapter 4

PROTOTYPING

Building the prototype was critical to assessing the feasibility of the proposed design. During prototyping, the unit continuously evolved as new ideas developed to improve the design. To establish technical feasibility one extraction column was completed, which is comprised of the extraction channel and elutriator. The initial prototype was constructed with modular parts to allow for easy modification of dimensions or positioning.

4.1 Elutriator

The initial elutriation design consisted of a 170 mm diameter reducer which are not as common as a 102 mm diameter PVC sanitary tee which was purchased at a hardware store. Additional PVC piping, a paint mixer and toilet flapper were also purchased to construct the elutriator. The 102 mm diameter PVC sanitary tee was connected to a 102x76 mm hub reducer coupling by 305 mm of PVC pipe. A 10 mesh was glued to the inner diameter of the sanitary tee. to maintain the 100 cc soil sample input, the elutriator length was increased resulting in the final length of the elutriator to be 355 mm. A pipe holder fastened the elutriator in position onto the frame. In accordance with the original design, the elutriator was placed 50 mm from the extraction boxes to allow clearance for pipes connecting to the jet sprays attached inside of the extraction boxes. Also, the outlet of the PVC sanitary tee must extend across the extraction box to ensure the overflow is carried onto the mesh surface. Additionally, a 60 mm clearance between the elutriator and the face of the extraction box was necessary to allot space for the egg extraction mechanism. The constructed elutriator presented some distinct changes from the

proposed design; instead of using greater diameters and smaller height, the length increased and diameters decreased. Nonetheless, elutriation can occur and space is still utilized efficiently. The rubber top cover was removed because the new dimensions of the elutriator will prevent soil sample spilling in contrast to the original wider design.

A toilet flapper was used to implement the lever mechanism responsible for sealing the elutriator. A 76 mm toilet flapper was attached to a 230 mm aluminum channel pivoted with a small shaft to the unit frame. Figure 4.3 shows how the channel was threaded to an additional shaft attached to a linear actuator. The channel was used as a lever to open and close the flapper as the linear actuator retracted and extended, respectively.

To provide water to the elutriator an overhead inlet has been added to the extraction unit. The shower head was centrally positioned 130 mm above the elutriator. A centrally located motor powers mixing bars via a roller chain. A gear train slows the 1700 RPM motor and is attached to a shaft that is connected to a roller chain to power all the mixing bars at the same time.

4.2 Extraction Box

The extraction boxes were assembled with ABS plastic sheets because it has a high impact reliance, stability, and cleaning ease. The extraction box was designed to stand at 170mm x 170 mm x 170 mm with a 15° sloped face that will hold the mesh screen. Custom, pre-cut ABS sheets were purchased from an online plastics company. The sheets, 3 mm thick, were glued together to construct the extraction box walls. The lip of the extraction box, purposed for transporting soil and water off the mesh and into the disposal system, were bent using a plastic heating tray, then glued to the extraction box.

The original prototype extraction box was assembled so the slope could be modified to test the most beneficial angle for cyst collection. At the 15° slope presented in the design, entire soil samples ran off the mesh screens quickly resulting in a considerable loss of cysts. Consequently, the slope was modified to 5° which improved control of water flow and cyst collection. Secondly, surface

tension on the meshes prevented water from going through the extraction boxes, which reduced water flow control and loss cysts intended for collection. By pre-wetting the extraction boxes the surface tension issues were resolved. As noted in Chapter 3, the design called for a stack of three extraction boxes per extraction column. The top extraction box, shown in Figure 4.4, was mounted to the frame due to constant pressure applied from the egg extraction mechanism. Accordingly, the top extraction box mesh was clamped to a perforated plate to increase roller stability and ensure the extracting mesh remained taut. The second extraction box did not require such restrictions to complete the task of collecting extraneous matter from egg samples and was glued to the extraction box. Both boxes were bolted onto the frame of the unit to provide stability and ensure the boxes remain aligned throughout the extraction process. The third extraction box, intended for egg sample collection, was replaced with a tipping plate. The tipping plate was beneficial because it was redundant to have an extraction box and a sample collection chamber accomplishing the same objective. Furthermore, the plate utilized less materials and space while achieving the process objectives. The tipping plate was created by bending each side of a rectangular ABS sheet to resemble a funnel on either side of the plate. The tipping plate was mounted to a pillow block and a shaft was threaded through the pillow block to allow motion between the two positions during the extraction process.

To clean the extraction boxes, a conical 210° angle jet spray was attached to the back wall of the box. As shown in Figure 3.3, the jets are attached to the angled lip.

4.2.1 Egg Extraction Mechanism

After elutriation, the cysts and some finer soil particles overflow to the first extraction box. The mesh on the extraction box holds cysts which are then crushed by a plastic roller. A 760 mm aluminum channel was assembled onto the metal drawer pulls. A linear actuator was mounted between the 760 mm aluminum channel attached to the drawer rails and a horizontal channel. The roller was spring loaded (see Figure 4.7) on a 230 mm aluminum channel mounted to the 760 mm channel to properly crush the cysts and maintain contact with the mesh screen.

4.3 Frame

The primary frame shown in Figure 4.8 was built to hold two complete extraction columns and the electric components required automation. Two sets of 380 mm aluminum channels were assembled as beams to serve as the base of the unit. The extraction box dimensions determined the placement of eight 710 mm support beams. The center support beams were attached perpendicular to the base beams and placed 200 mm from each end of the base beams. An additional 380 mm aluminum channel was attached to the center beams to provide a mounting point for the egg extraction mechanism. A secondary frame supports the elutriator, overhead water outlet piping, and the elutriator sealing assembly. The secondary frame is 1145 mm high and 760 mm wide. The elutriator sealing assembly is mounted similar to the egg extraction mechanism. The actuator is mounted on a stationary aluminum channel and the rod is mounted to a 38 mm channel attached to the shaft.

The center 200 mm space of the unit was allotted for electronic components to provide easier access for trouble shooting and protection from potential water damage. Because of the nature of SCN extraction and the use of a considerable amount of water, the inner section of the frame reserved for electronics would be protected with a barrier. Even though the prototype frame was built to fit only two extraction columns, the design is scalable. All of the automated components were assembled so additional columns can readily be extended on either side of the foundational unit without purchasing more electronic control parts.

4.4 Continued Prototyping

Prototyping the extraction unit revealed limitations within the original design. The first prototype has resulted in a secondary design process to improve specifications to maximize extraction efficiency. The unit continuously undergoes modification which will surpass the time to complete this thesis. Remaining prototyping to be accomplished includes: elutriator inlet location, electronic component placement inside the allotted space, electric wiring, and waste disposal system. The

extraction unit design (see Figure 4.9) has been updated to reflect changes made during the initial prototype process.

Figure 4.1: Prototype Extraction Unit.

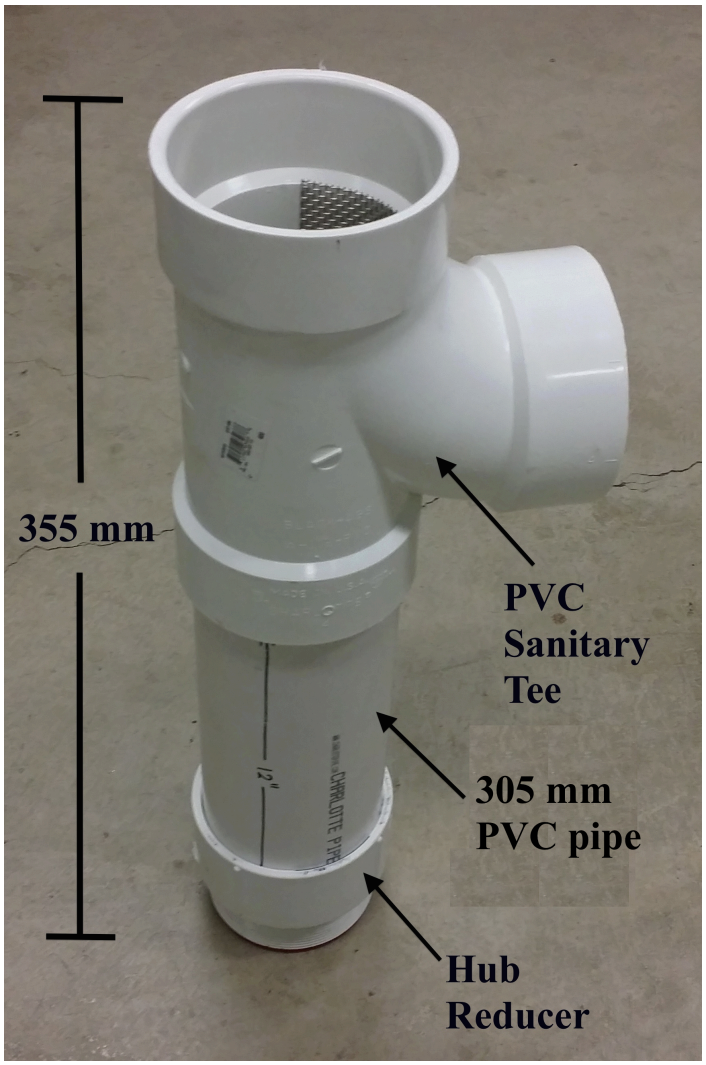

Figure 4.2: Constructed Elutriator. The dimensions are modified from the original design in Figure 3.2, yet elutriation can occur.

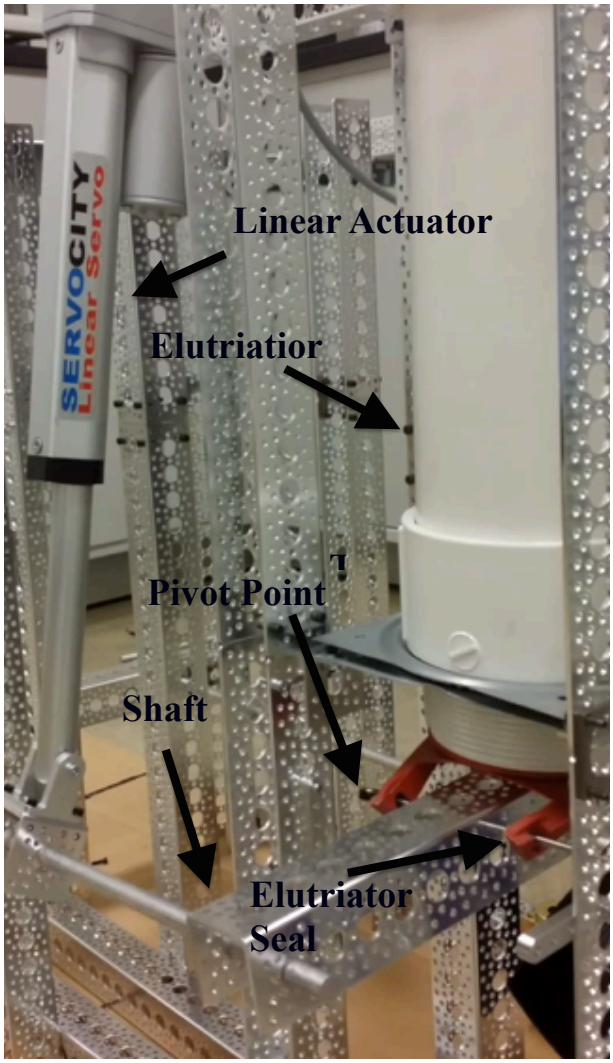

Figure 4.3: Elutriator Sealing Mechanism. The channel is attached to the unit frame by a pivot point. An additional shaft is threaded through a linear actuator and the channel to move the flapper.

Figure 4.4: The top extraction box. The mesh screen is attached to the frame of the unit to provide stability during extraction. The extraction box below does not require the mesh to be as rigidly fixed like the first box.

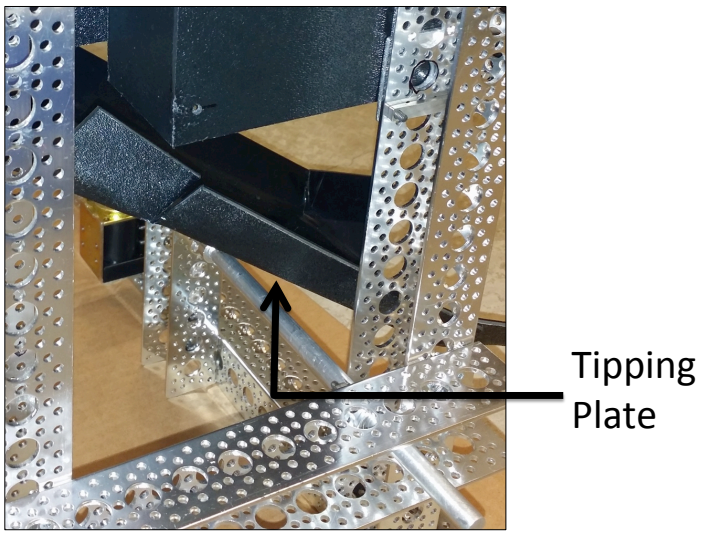

Figure 4.5: New tipping plate to replace the third extraction box. The tipping plate helps to reduce material use by eliminating the stainless steel mesh and reduced use of plastic ABS sheets.

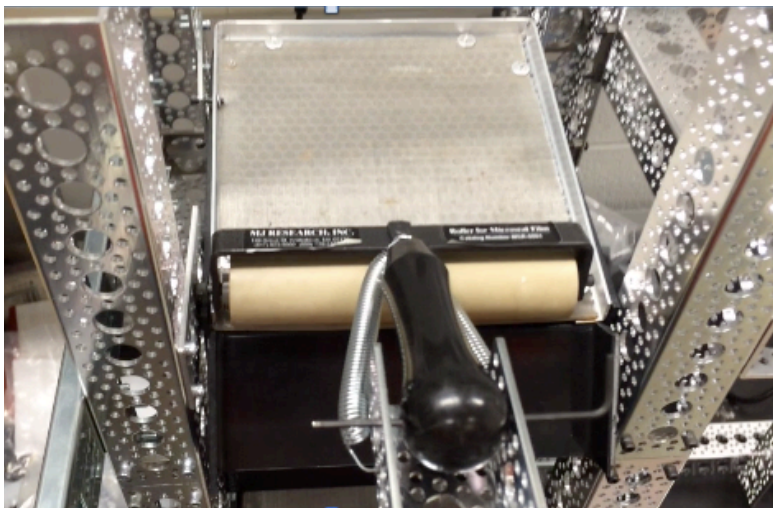

Figure 4.6: Prototyped roller mechanism. The plastic roller is spring loaded in order to remain flush to the mesh screen

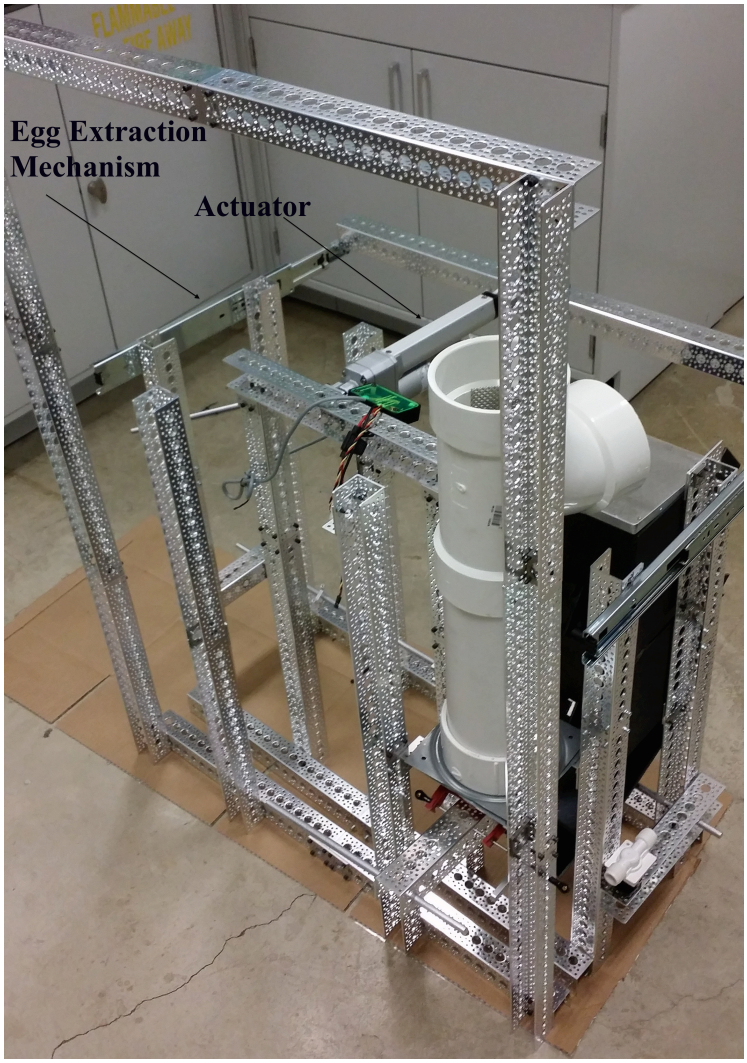

Figure 4.7: Prototyped roller mechanism. The linear actuator is mounted to a support channel in the center of the unit. The drawer rails are placed on the outside of the extraction unit.

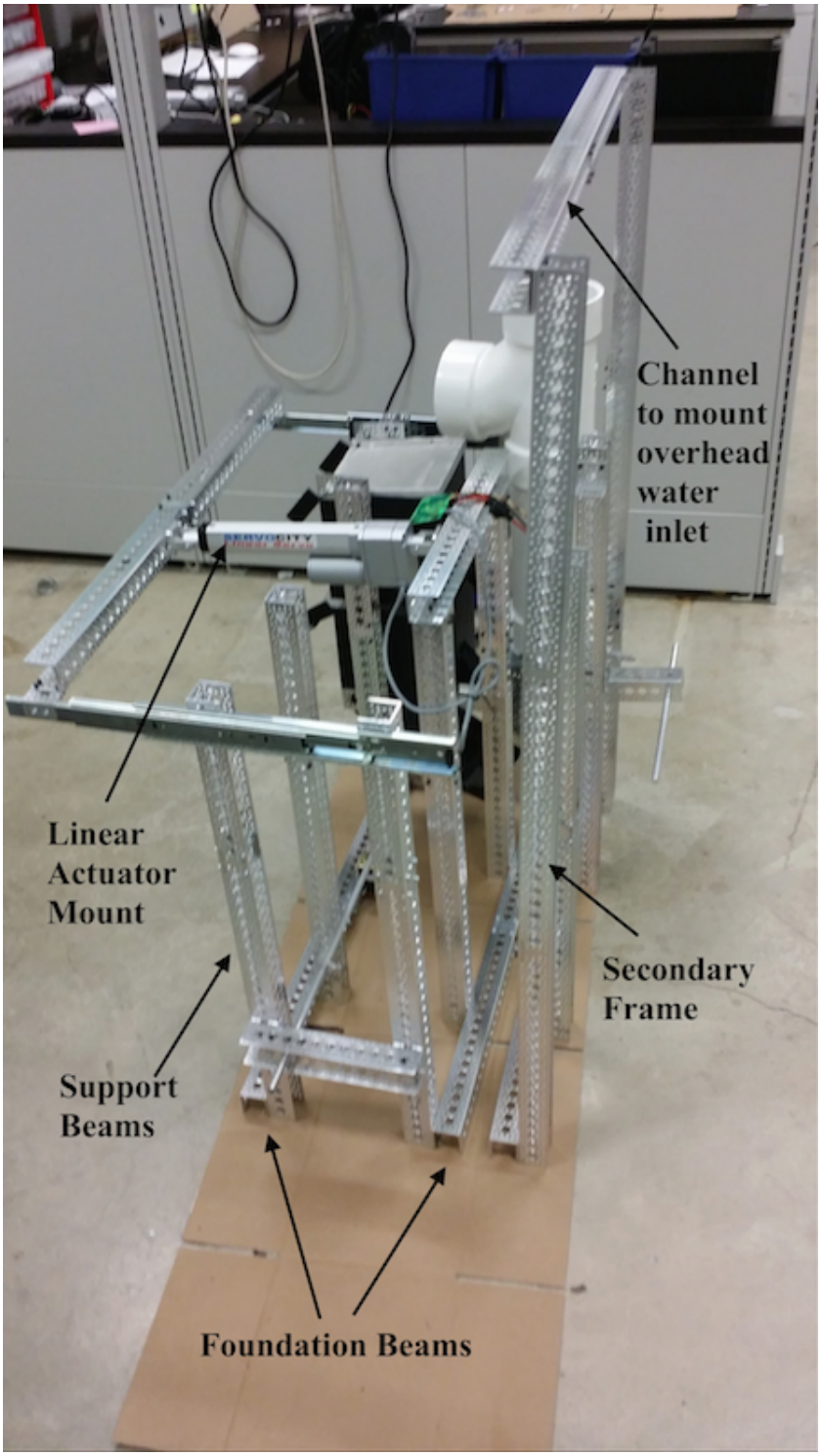

Figure 4.8: Prototype Extraction Unit Frame.

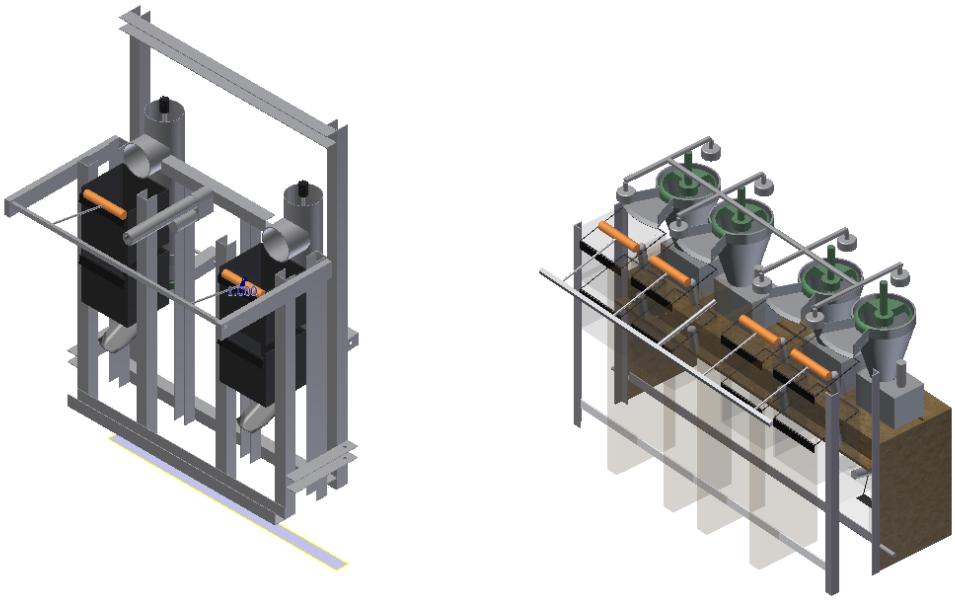

Figure 4.9: Left: Second iteration SCN extraction unit design. Right: First iteration SCN extraction unit design.

Chapter 5

AUTOMATION

Automation is a critical component to achieving a high extraction efficiency. Implementing an automated process will reduce sampling time, improve cyst recovery rates, and achieve robustness to soil variability. Cyst recovery rate increases by the elimination of handling variability. Operators testing soil for SCN inherently introduce variability. Any error that may arise from the automated extraction process will be less than that of an operator and expected remain constant. Most of the current SCN extraction methods are not able to maintain the typical recovery rate for soil types. Moreover, the techniques are amenable to large scale production and suffer from inconsistent recovery rates due to soil type. Here, a new design is presented that can provide robustness to soil variability, in addition to eliminating the soil homogenization step. Also, the automated mixing component provides the force to quickly and effectively overcome variable recovery rates typically associated with soil types.

This chapter explores the steps and components to be implemented in order to achieve automation of the SCN extraction unit. Due to the iterative nature of prototyping, automation needs also continuously evolve. For this reason, the code is continuously under evaluation. However, the identification of electronic components to achieve automation proves that it is feasible when the prototype is complete.

5.1 Automation Scheme

5.1.1 Soil Sample Preparation

Prior to depositing a soil sample, a small sequence of automated steps occur:

- Pre-wet sieves
- Close the elutriator seal
- Position roller at the base of the extraction box
- Move tipping plate to the waste discharge position

First, as mentioned in 4.2, the sieves are pre-wetted to reduce surface tension. The overhead water inlet flow is regulated by a solenoid control valve that releases water onto the extraction channel. Next, the elutriator seal assembly is closed to contain a soil sample. A linear actuator extends to pivot the assembly closed as discussed in 4.1. Afterwards, the roller is positioned at the base of the extraction box as shown in Figure 4.7. A solenoid tipped the plate forward to direct any finer particulates from elutriation to the disposal system.

5.1.2 Elutriation

After a sample has been deposited, automated elutriation can be accomplished with the following:

- Fill elutriator with 2-3 L of water
- Mix soil sample

    Water inlet continue to fill elutriator to produce outlet overflow

- Open elutriator seal to release soil sample

Water is released by a solenoid control valve to fill the tank. The mixing bar, powered by the motor mixes the soil sample. A solenoid control valve releases water through the elutriator inlet to produce the overflow that carries cysts and

fine soil particles to the extraction channel. When elutriation is complete the actuator attached to the seal assembly will retract to discharge the soil sample.

5.1.3 Egg Extraction and Collection

SCN eggs are extracted and collected by the completion of these steps.

- Crush cysts
- Transport eggs through the extraction channel and direct debris to disposal system
- Collect egg sample
- Transport the sample to a collection chamber

The cysts are crushed by the extension and retraction of the linear actuator which drives the roller. Overhead water inlets, controlled by solenoid control valves, provide water flow to facilitate the transportation of eggs and remaining debris to the second extraction box. The water from the inlet carries the eggs onto the tipping collection plate. After elutriation, a solenoid moves the tipping plate position to direct eggs into a collection chamber.

5.1.4 Unit Cleaning

Self-cleaning can be accomplished by addressing the list below.

- Open elutriator seal assembly
- Clean elutriator
- Clean extraction channel

The elutriator seal is driven open by the extension of a linear actuator. Solenoid control valves release water to the conical jet sprays inside the extraction boxes for cleaning and the overhead water inlet to clean the elutriator.

5.2 Remaining Automation

As noted before, some code has been developed but is still under evaluation at the time of thesis deposit. The code to control the motor driving the mixing bar mechanism bar, the solenoid valve for the tipping plate, and solenoid valves that will control water flow has yet to be completed.

Chapter 6

DISCUSSION

The automated extraction unit should work because all high SCN extraction efficiency can achieved by one of the three components of the unit. The process has been consolidated effectively, the unit serves as proof that the concept is feasible, and the automation can be accomplished.

As a result, operator-related variance is eliminated and farm SCN infestation diagnosis reliability will improve. Automation eradicates fluctuating recovery rates and establishes the uniformity of cyst recovery rate. Accordingly, SCN infestation diagnosis will become more accurate, consequently improving nematode management plans.

Laboratory bench space is typically prime in any lab; some labs perform SCN egg extraction in a separate space. The automation unit proposed in this paper is modular; the minimal two-channel unit utilizes a mere 30 inches of bench space and stands approximately 3 feet high.

Data reliability is critical for monitoring SCN populations. The automation will improve data reliability and may assist in standardizing SCN egg population recovery in soil samples. Automated SCN egg extraction has many benefits that will positively impact the study of nematology. The use of open source software and moderately challenging fabrication makes it a viable option for any level of SCN research activity.

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